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ORIGINAL ARTICLE

Plasma homocysteine and folate levels and dietary folate intake in adolescents and young adults who underwent kidney transplantation during childhood

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Abstract
Background Hyperhomocysteinemia (hyper-Hcy) is an important and reversible cardiovascular disease risk factor. We examined the prevalence of hyper-Hcy, plasma folate levels, and dietary folate intake in adolescents and young adults who had undergone kidney transplantation during childhood to assess the necessity for managing dietary folate.
Methods This cross-sectional study was performed in 89 kidney transplant recipients (age at kidney transplantation: 12.6 ± 4.1 years; age during study: 21.2 ± 5.5 years). Hyper-Hcy and plasma folate deficiency were defined as plasma homocysteine (Hcy) >15 nmol/ml and plasma folate <3.0 ng/ml, respectively.
Results Of the patients, 60 (67.4 %) had hyper-Hcy and 14 (15.7 %) had plasma folate deficiency. Plasma homocysteine levels correlated negatively with estimated glomerular filtration rate (eGFR; $r = -0.565$, $p < 0.01$) and plasma folate levels ($r = -0.434$, $p < 0.01$). For determinants of plasma homocysteine levels, a priori selected variables included kind of calcineurin inhibitor, age at kidney transplantation, pretransplant duration of dialysis, time since transplantation, age at examination, eGFR, and plasma folate. Stepwise multiple linear regression analysis revealed eGFR and plasma folate levels as significant independent variables influencing plasma homocysteine levels. Dietary folate intake in 11 of 16 patients (66.8 %) with eGFR ≥60 ml/min/1.73 m^2 was below the recommended dietary allowance for Japanese.
Conclusions The prevalence of hyper-Hcy and plasma folate deficiency, as well as the low dietary folate intake, suggest that dietary management of folate is necessary for adolescents and young adults who have undergone kidney transplantation during childhood.

Keywords Pediatric kidney transplantation · Adolescent and young adult kidney transplant recipients · Homocysteine · Folate · Dietary management

Introduction

Cardiovascular disease (CVD) is common in kidney transplant recipients [1]. Similar to in adults, CVD morbidity and mortality in pediatric kidney transplant recipients are higher than in the general population [2]. Therefore, primary prevention and treatment of CVD are considerations for the management of kidney transplant recipients for long-term survival following transplantation. CVD morbidity and mortality are caused by the presence of traditional risk factors, such as hypertension, dyslipidemia, and diabetes mellitus, and are compounded by several nontraditional risk factors, such as calcium-phosphate metabolism, hyperparathyroidism, and inflammation [2]. Hyperhomocysteinemia (hyper-Hcy) has been identified as an important and reversible CVD risk factor [3] that is

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commonly found in chronic kidney disease (CKD) patients [4]. In hyper-Hcy, plasma homocysteine levels tend to increase in line with decreasing glomerular filtration rate (GFR) [5]. In addition to kidney dysfunction, folate intake can also influence plasma homocysteine levels [4].

The prevalence of hyper-Hcy and the relationship between plasma homocysteine and plasma folate levels are well known in adult kidney transplant recipients [6]. However, little is known on this relationship in adolescents and young adults who have undergone kidney transplant as a child [7]. Moreover, there are few reports of dietary folate intake in kidney transplant recipients of any age. Therefore, we examined the prevalence of hyper-Hcy, plasma folate levels, and dietary folate intake in adolescents and young adults who had undergone kidney transplantation during childhood to assess the necessity for managing dietary folate.

Patients and methods

Patients and plasma samples

The study was approved by the ethics committee of the School of Medicine at Tokyo Women's Medical University (approval number 2445). This cross-sectional study was performed in 89 adolescent and young adult recipients who had undergone kidney transplantation during childhood. No patients were on folic acid supplementation before and during the data collection period (October to November, 2009). Patient data included age at the time of the study, sex, primary disease, transplant characteristics, donor difference (living or cadaver), preemptive kidney transplantation (PEKT) or nonpreemptive kidney transplantation (non-PEKT), pretransplant duration of dialysis, age at kidney transplantation, time since transplantation, and frequency of calcineurin inhibitor (tacrolimus or cyclosporine) use.

The estimated glomerular filtration rate (eGFR) of young adults was calculated using the Japanese equation for estimating GFR [8], while the eGFR of adolescents was assessed according to Schwartz's formula [9]. Plasma homocysteine was measured by high-performance liquid chromatography [10], and plasma folate was measured by chemiluminescent enzyme immunoassay (CLEIA) [11]. Both procedures were conducted by SRL, Inc. (Tokyo, Japan).

eGFR level was defined as follows: normal, $\geq$90 ml/min/1.73 m^2; mild decreased, $\geq$60 and $<$90 ml/min/1.73 m^2; and moderate to severe decreased, $<$60 ml/min/1.73 m^2 [12]. Hyper-Hcy was defined as homocysteine $>$15 nmol/ml [13]. Plasma folate deficiency was defined as plasma folate $<$3.0 ng/ml [14].

Dietary folate intake

Dietary folate intake was measured using the food frequency questionnaires (FFQg) method [15] that included 29 food groups and 10 kinds of cooking methods. The questionnaire was designed to estimate the energy and nutrient intake of the respondent during the previous 1–2 months. The reproducibility and validity of this method are well accepted [15]. The recommended dietary allowance (RDA) of folate is 240 μg/day [16], and the RDA was used to evaluate whether or not the dietary folate intake of patients was sufficient.

Statistical analysis

Statistical analysis was performed using SPSS version 12.0 (SPSS Inc., Chicago, IL, USA). Data are presented as mean ± standard deviation (SD), median, and range values. The Kolmogorov–Smirnov test was used to test normality, and logarithmic transformations were made for all variables, if needed. Spearman's rank correlation coefficients were used to analyze the association between plasma homocysteine and eGFR or plasma folate levels. Stepwise multiple linear regression models were used to examine the determinants of plasma homocysteine levels. The statistical difference of dietary folate intake between the two groups was determined using the Mann–Whitney U test. Significance was set at $p < 0.05$.

Results

Clinical characteristics of patients

Adolescents and young adults [$n = 89$; male, $n = 48$ (53.9 %)] who had undergone kidney transplantation during childhood were examined. Clinical characteristics of patients are presented in Table 1. Congenital anomalies of the kidney and urinary tract (CAKUT) was the most common primary disease (47.2 %), and living kidney donors constituted 84.3 % ($n = 75$). Patients who had received kidney transplantation after dialysis accounted for 78.7 % of subjects, and the mean pretransplant duration of dialysis was 3.1 ± 2.5 years. The mean age at kidney transplantation was 12.6 ± 4.1 years, and the mean time since transplantation was 9.0 ± 5.7 years. The frequency of tacrolimus use was 75.3 %. The mean age at the time of the study was 21.2 ± 5.5 years, and the mean eGFR of patients was 55.5 ± 19.9 ml/min/1.73 m^2.

Plasma homocysteine distribution

The distribution of plasma homocysteine levels (7.2–69.3 nmol/ml, mean 20.8 ± 12.1 nmol/ml) is shown in Fig. 1. Sixty patients (67.4 %) had hyper-Hcy.

Table 1 Characteristics of kidney transplant recipients ($n = 89$)

Variable	n (%) or mean ± SD	Median	Range
Gender			
Male	48 (53.9)		
Female	41 (46.1)		
Primary disease			
CAKUT	42 (47.2)		
FSGS	16 (18.0)		
GN	14 (15.7)		
CNS	2 (2.2)		
Others	15 (16.9)		
Kidney transplantation			
Living kidney transplantation	75 (84.3)		
Cadaver kidney transplantation	14 (15.7)		
Preemptive transplantation	19 (21.3)		
Nonpreemptive transplantation	70 (78.7)		
Pretransplant duration of dialysis (years)	3.1 ± 2.5	2.5	0.2–10.6
Age at transplantation (years)	12.6 ± 4.1	13	3.7–19.8
Years posttransplant (years)	9.0 ± 5.7	8.3	0.3–27.3
Calcineurin inhibitor			
Tacrolimus	67 (75.3)		
Cyclosporine	21 (23.6)		
Patient characteristics examined at the study			
Age (years)	21.2 ± 5.5	20	15–38
eGFR (ml/min/1.73 m^2)	55.5 ± 19.9	55	8–102
eGFR ≥90	4 (4.5)		
60 ≤ eGFR < 90	33 (58.4)		
eGFR < 60	52 (37.1)		

Data presented as percentage, mean ± standard deviation (SD), median, and range

BMI body mass index, *eGFR* estimated glomerular filtration rate, *CAKUT* congenital anomalies of the kidney and urinary tract, *GN* glomerulonephritis, *FSGS* focal segmental glomerulosclerosis, *CNS* congenital nephrotic syndrome

Plasma folate distribution

The distribution of plasma folate levels (1.9–15.2 ng/ml, mean 4.8 ± 2.3 ng/ml) is shown in Fig. 2. Fourteen patients (15.7 %) had plasma folate deficiency.

Plasma homocysteine and eGFR

Plasma homocysteine levels correlated negatively with eGFR ($r = -0.565$, $p < 0.01$) (Fig. 3).

Plasma homocysteine and plasma folate

Plasma homocysteine levels correlated negatively with plasma folate levels ($r = -0.434$, $p < 0.01$) (Fig. 4).

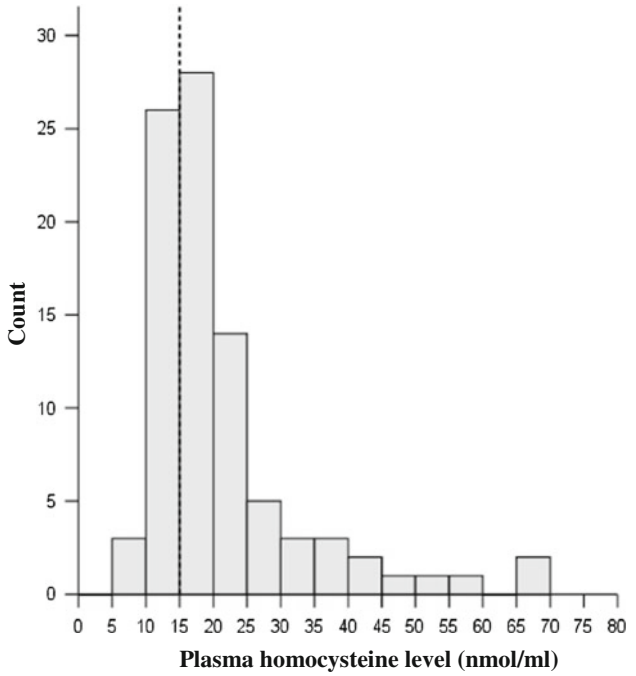

Fig. 1 Plasma homocysteine (Hcy) levels. The *dotted line* indicates the normal Hcy level (15.0 nmol/ml). Sixty of 89 patients (67.4 %) had hyperhomocysteinemia (hyper-Hcy)

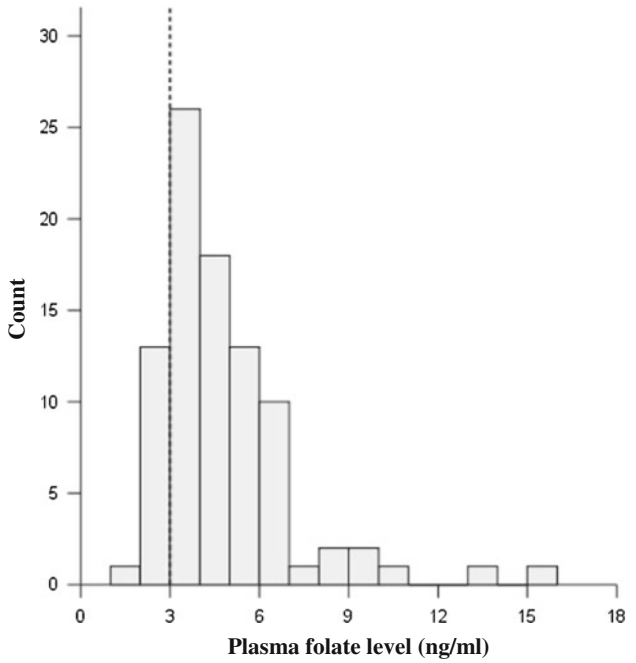

Fig. 2 Plasma folate levels. The *dotted line* indicates the normal folate level (3.0 ng/ml). Fourteen of 89 patients (15.7 %) had plasma folate deficiency

Determinants of plasma homocysteine

For determinants of plasma homocysteine levels, a priori selected variables included kind of calcineurin inhibitor

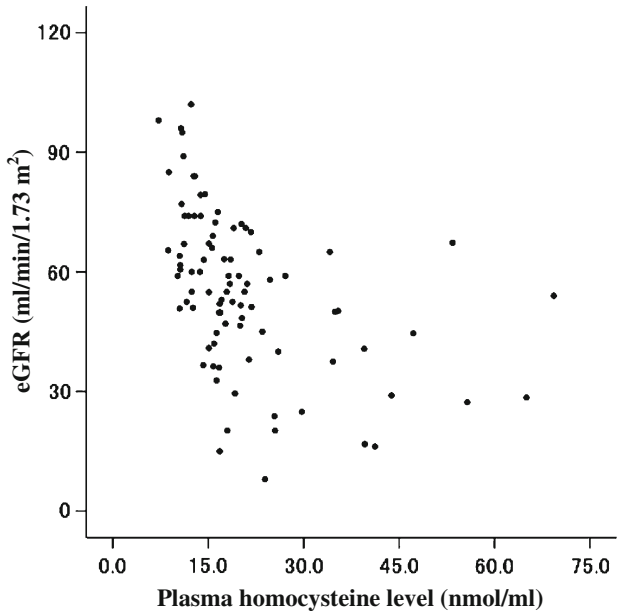

Fig. 3 Relationship between eGFR and plasma homocysteine (Hcy) levels. Plasma Hcy levels correlate negatively with eGFR ($r = -0.565$, $p < 0.01$)

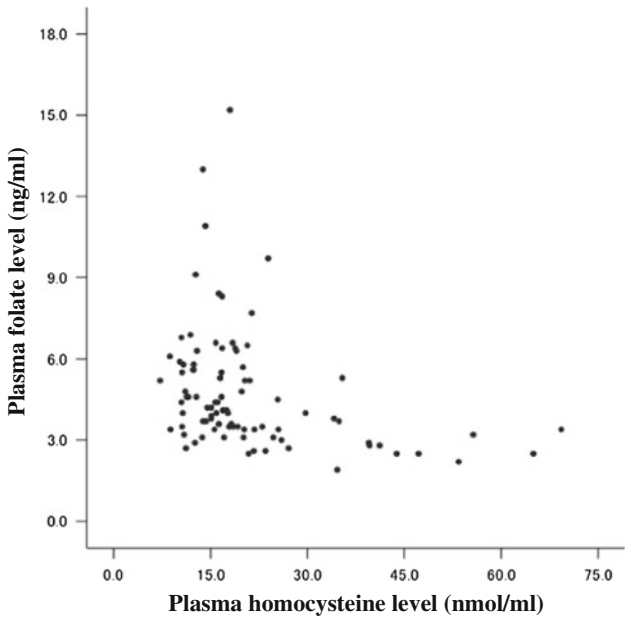

Fig. 4 Relationship between plasma folate levels and plasma homocysteine (Hcy) levels. Plasma Hcy levels correlate negatively with plasma folate levels ($r = -0.434$, $p < 0.01$)

(tacrolimus or cyclosporine), age at kidney transplantation, pretransplant duration of dialysis, time since transplantation, age at examination, eGFR, and plasma folate. Stepwise multiple linear regression analysis revealed eGFR ($\beta = -0.522$, $t = -6.521$, $p < 0.001$) and plasma folate ($\beta = -0.453$, $t = -5.667$, $p < 0.001$) levels as significant independent variables influencing plasma homocysteine levels (adjusted $R^2 = 0.439$).

Dietary folate intake

Depending on the residual kidney function, individual dietary restrictions may be needed in CKD patients. Patients with decreased eGFR were likely to have supervised dietary restrictions. In addition, patients with moderate to severe decreased eGFR tended to take sevelamer hydrochloride, which interferes with the absorption of dietary folate [17]. Therefore, dietary folate intake was examined in 37 patients with eGFR ≥60 ml/min/1.73 m^2, and valid data were collected from 16 of these patients (43.2 %). Dietary folate intake was 104–297 μg/day, and mean dietary folate intake was 196.9 ± 58.0 μg/day. Dietary folate intake in 11 of the 16 patients (66.8 %) was below the RDA for Japanese.

To determine the reasons for dietary folate deficiency, several clinical features were compared between the PEKT group ($n = 5$) and the non-PEKT group ($n = 11$). No differences were noted between the two groups for age and eGFR during the study, time since kidney transplantation, and energy intake (data not shown). Although no significant difference between the two groups was detected, dietary folate intake in the non-PEKT group (179.7 ± 51.9 μg/day, median 159 μg/day) had a tendency to be lower than that in the PEKT group (234.7 ± 57.5 μg/day, median 254 μg/day) ($p = 0.157$).

Discussion

Hyper-Hcy was prevalent (67.4 %) in adolescents and young adults who had undergone kidney transplantation during childhood. Becker-Cohen et al. [18] reported that 58 % (35 of 60) of children and young adult kidney transplant recipients had hyper-Hcy. Similarly, Belson et al. [19] showed that 77 % (54 of 70) of pediatric and young adult kidney transplant recipients had hyper-Hcy. Therefore, our results are in agreement with these previous studies [18, 19]. Thus, hyper-Hcy is common in adolescents and young adults who have undergone kidney transplantation during childhood.

Kidney tissue contains Hcy-metabolizing enzymes such as methionine synthase and cystathionine β-synthase. High methionine synthase activity is found in the liver and kidney, and cystathionine β-synthase expression is detected in kidney proximal tubular cells [4]. Kidney tissue plays a role in homocysteine clearance and metabolism [5]. Previous studies have shown that patients with decreased GFR are more likely to have hyper-Hcy [6, 19]. Our study showed that plasma homocysteine levels correlate

negatively with eGFR, which is in agreement with the results of previous studies [6, 19]. The quality of posttransplant kidney function correlates significantly with CVD risk [1]. Thus, our study suggests that preserving posttransplant residual kidney function has an impact on improving plasma homocysteine levels and reducing several CVD risk.

In addition to kidney dysfunction, homocysteine metabolism is influenced by plasma folate levels [4]. Our study showed that 15.7 % of patients (14 of 89) had plasma folate deficiency. Sakuta et al. [20] reported that gastrectomized patients have a predisposition for plasma folate deficiency and that the appearance of plasma folate deficiency is higher in gastrectomized patients (12.9 %) compared with healthy subjects (0 %). Given these figures, the prevalence of folate deficiency in the present study seemed to be on a par with that of gastrectomized patients.

An inverse association between plasma homocysteine and plasma folate levels was reported in some previous studies [4, 10]. Our study showed that plasma homocysteine levels correlate negatively with plasma folate levels, which is in agreement with the results of previous studies [4, 10]. To examine factors other than eGFR and plasma folate that influence plasma homocysteine levels, we performed stepwise multiple linear regression analysis. A priori selected variables included kind of calcineurin inhibitor, age at kidney transplantation, pretransplant duration of dialysis, time since transplantation, and age at examination. Stepwise multiple linear regression analysis revealed eGFR and plasma folate levels as significant independent variables influencing plasma homocysteine levels.

Since dietary folate intake influences plasma folate levels [21], dietary management of folate is important to control plasma homocysteine levels after kidney transplantation.

Brouwer et al. [22] reported that dietary folate from vegetables and citrus fruits improves plasma folate status and decreases plasma homocysteine concentrations. In this study, dietary folate intake in 66.8 % of patients (11 of 16) was below the RDA for Japanese. Watanabe et al. [23] reported that dietary folate deficiency is associated with depression in Japanese women aged 18–29 years and that the prevalence of dietary folate deficiency (<RDA) was significantly higher in subjects with depression (75.0 %) compared with healthy subjects (43.6 %). Although it is difficult to make a simple comparison of the prevalence of dietary folate deficiency with this previous study [23], the prevalence of dietary folate deficiency seems to be high in the present study.

The reasons for the high number of patients with poor folate intake in this study are unknown. Vegetables such as spinach, asparagus, broccoli, and potato and citrus fruits such as orange are good sources of folate. Patients on dialysis have limited intake of these foods because of dietary potassium restrictions. Here, dietary folate intake in the non-PEKT group had a tendency to be lower than that in the PEKT group. Given this result, it is speculated that dietary restrictions, including folate-containing foodstuff restrictions, in the pretransplant dialysis period were continued after kidney transplantation in the non-PEKT group. Although further studies are clearly necessary to verify this speculation, our results suggest that appropriate dietary management for adequate dietary folate intake is necessary for kidney transplant recipients.

Finally, the efficacy of high folate intake for lowering plasma homocysteine levels in an effort to reduce CVD risk in kidney transplant recipients remains inconclusive [4]. A prospective approach is needed to study the effects of managing dietary folate on CVD morbidity and mortality in adolescents and young adults who have undergone kidney transplantation during childhood.

Conclusions

Hyper-Hcy and plasma folate deficiency were highly prevalent in adolescents and young adults who have undergone kidney transplantation during childhood. Moreover, dietary folate intake in many patients was below the RDA for Japanese. Taken together, the present findings suggest that dietary management of folate is necessary for adolescents and young adults who have undergone kidney transplantation during childhood.

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Conflict of interest The authors have declared that no conflict of interest exists.

References

1. Liefeldt L, Budde K. Risk factors for cardiovascular disease in renal transplant recipients and strategies to minimize risk. Transplant Int. 2010;23:1191–204.
2. Dégi A, Kerti A, Kis E, Cseprekál O, Tory K, Szabó AJ, Reusz GS. Cardiovascular risk assessment in children following kidney transplantation. Pediatr Transplant. 2012;16:564–76.
3. Clarke R, Daly L, Robinson K, Naughten E, Cahalane S, Fowler B, Graham I. Hyperhomocysteinemia: an independent risk factor for vascular disease. N Engl J Med. 1991;324:1149–55.
4. Qin X, Huo Y, Langman CB, Hou F, Chen Y, Matossian D, Xu X, Wang X. Folic acid therapy and cardiovascular disease in ESRD or advanced chronic kidney disease: a meta-analysis. Clin J Am Soc Nephrol. 2011;6:482–8.

5. Friedman AN, Bostom AG, Selhub J, Levey AS, Rosenberg IH. The kidney and homocysteine metabolism. J Am Soc Nephrol. 2001;12:2181–9.
6. Winkelmayer WC, Kramar R, Curhan GC, Chandraker A, Endler G, Födinger M, Hörl WH, Sunder-Plassmann G. Fasting plasma total homocysteine levels and mortality and allograft loss in kidney transplant recipients: a prospective study. J Am Soc Nephrol. 2005;16:255–60.
7. Merouani A, Delvin EE, Genest J Jr, Rozen R, Lambert M. Plasma homocysteine concentration changes after renal transplantation in children. Pediatr Nephrol. 2002;17:520–3.
8. Matsuo S, Imai E, Horio M, Yasuda Y, Tomita K, Nitta K, Yamagata K, Tomino Y, Yokoyama H, Hishida A, Collaborators developing the Japanese equation for estimated GFR. Revised equations for estimated GFR from serum creatinine in Japan. Am J Kidney Dis. 2009;53:982–92.
9. Schwartz GJ, Brion LP, Spitzer A. The use of plasma creatinine concentration for estimating glomerular filtration rate in infants, children, and adolescents. Pediatr Clin North Am. 1987;34:571–90.
10. Menon V, Wang X, Greene T, Beck GJ, Kusek JW, Selhub J, Levey AS, Sarnak MJ. Homocysteine in chronic kidney disease: effect of low protein diet and repletion with B vitamins. Kidney Int. 2005;67:1539–46.
11. Shiraishi M, Haruna M, Matsuzaki M, Ota E, Murayama R, Murashima S. Association between the serum folate levels and tea consumption during pregnancy. Biosci Trends. 2010;4:225–30.
12. National Kidney Foundation. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. Am J Kidney Dis. 2002;39:S1–266.
13. Kazemi MB, Eshraghian K, Omrani GR, Lankarani KB, Hosseini E. Homocysteine level and coronary artery disease. Angiology. 2006;57:9–14.
14. Shane B. Folate status assessment history: implications for measurement of biomarkers in NHANES. Am J Clin Nutr. 2011;94:337S–42S.
15. Yoshimura Y, Kamada C, Takahashi K, Kaimoto T, Iimuro S, Ohashi Y, Araki A, Umegaki H, Sakurai T, Ito H; Japanese Elderly Diabetes Intervention Trial Study Group. Relations of nutritional intake to age, sex and body mass index in Japanese elderly patients with type 2 diabetes: the Japanese Elderly Diabetes Intervention Trial. Geriatr Gerontol Int. 2012; 12 Suppl 1:29–40. doi: 10.1111/j.1447-0594.2011.00810.x.
16. Ministry of Health, Labour and Welfare. Dietary Reference Intakes for Japanese (2010). 2009; 162–4 (In Japanese).
17. Goto S, Fujii H, Kim JI, Fukagawa M. Homocysteine and folic acid levels in hemodialysis patients treated with sevelamer hydrochloride. Clin Nephrol. 2010;73:420–5.
18. Becker-Cohen R, Nir A, Rinat C, Feinstein S, Algur N, Farber B, Frishberg Y. Risk factors for cardiovascular disease in children and young adults after renal transplantation. Clin J Am Soc Nephrol. 2006;1:1284–92.
19. Belson A, Sanchez J, Alexander SR, Salvatierra O, Dar MH, Reif S, Yorgin PD. Hyperhomocysteinemia in pediatric and young adult renal transplant recipients. Pediatr Transplant. 2004;8:161–6.
20. Sakuta H, Suzuki T, Yasuda H, Wakiyama H, Hase K. Plasma vitamin B12, folate and homocysteine levels in gastrectomized men. Clin Nutr. 2005;24:244–9.
21. Milne DB, Johnson LK, Mahalko JR, Sandstead HH. Folate status of adult males living in a metabolic unit: possible relationships with iron nutriture. Am J Clin Nutr. 1983;37:768–73.
22. Brouwer IA, van Dusseldorp M, West CE, Meyboom S, Thomas CM, Duran M, van het Hof KH, Eskes TK, Hautvast JG, Steegers-Theunissen RP. Dietary folate from vegetables and citrus fruit decreases plasma homocysteine concentrations in humans in a dietary controlled trial. J Nutr. 1999;129:1135–9.
23. Watanabe H, Ishida S, Konno Y, Matsumoto M, Nomachi S, Masaki K, Okayama H, Nagai Y. Impact of dietary folate intake on depressive symptoms in young women of reproductive age. J Midwifery Womens Health. 2012;57:43–8.